

CLAIMS

1. A method of qualitative and quantitative analysis of the microbial population(s) potentially present in a sample, characterized in that it comprises:
- 5 - contacting the microorganisms potentially present in said sample with at least one RNA-targeted oligonucleotide probe, hereafter called specific probe, able to target a desired microbiological population, under conditions favourable to *in situ* hybridization in whole cells,
- 10 - extracting by separation from their target and elution outside said cells those probes which have become hybridized,
- detecting the extracted probes and measuring the amount thereof or their respective amounts.
- 15 2. A method according to Claim 1, further characterized in that said at least one specific probe is chosen among the group consisting of Nb 1000 (SEQ ID N°1) and Nso 1225 (SEQ ID N°2).
- 20 3. A method according to Claim 1 or 2, further characterized in that said microorganisms potentially present in said sample are contacted with another probe, hereafter called universal probe, serving to normalize results obtained with probes targeting specific phylogenetic groups of microorganisms.
- 25 4. A method according to Claim 3, further characterized in that said universal probe is chosen among the group consisting of S Univ-1390 (SEQ ID N°3) and S Bac 338 (SEQ ID N°4).
- 30 5. A method according to any one of the preceding claims, further characterized in that said specific and/or universal probe(s) is a (are) rRNA-targeted probe(s).

a 6. A method according to any one of the preceding Claim 1 ~~Claims~~, further characterized in that said microorganisms potentially present in said sample are extracted from said sample, notably by centrifugation.

a 5 7. A method according to any one of the preceding Claim 1 ~~claims~~, further characterized in that said contacting is performed following fixation of said cells.

10 8. A method according to claim 7, further characterized in that said fixation of the cells is achieved by incubation of said cells in a solution of less than 10% paraformaldehyde, and preferably about 4%, for 3 to 12 hours at 4°C.

a 15 9. A method according to claim 7 or 8, further characterized in that said fixation is followed by a dehydration step, prior to said contacting step.

20 10. A method according to claim 9, further characterized in that said dehydration step is performed by placing said sample in contact with at least one ethanol solution, and preferably with a series of ethanol solutions of increasing concentrations.

a 25 11. A method according to any one of the preceding Claim 1 ~~claims~~, further characterized in that said contacting is performed by placing said sample in contact with said at least one probe in the presence of a solution, hereafter called hybridization solution, which notably comprises a denaturing agent such as sodium dodecyl sulfate (SDS) at a concentration in a 0,001-0,1% range, preferably on the order of 0.01%, Tris-HCl, pH of about 8 at a concentration in a 0,001-0,1 M range, preferably on the order of 0.02M; and 30 a salt such as sodium chloride at a concentration in a 0,1-1,5 M range, preferably on the order of 0.9 M.

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12. A method according to any one of the preceding ~~Claims~~ ^{Claim 1}, further characterized in that said contacting phase is performed for an incubation time of about 10 minutes to about 2 hours, and at the optimal hybridization temperature.

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13. A method according to any one of the preceding ~~claims~~ ^{Claim 1}, further characterized in that said extraction of said at least one probe is performed following removal of the excess and unbound probe or of non-specifically associated probe material placed in contact, notably by washing with a solution, hereafter called wash solution, which notably comprises a denaturing agent such as sodium dodecyl sulfate (SDS) and a salt such as sodium chloride at concentrations appropriate for achieving the stringency necessary to the removal of non-specifically associated probe.

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14. A method according to any one of the preceding ~~claims~~ ^{Claim 1}, further characterized in that said extraction is performed by placing said microorganisms potentially present under conditions enabling the denaturation of every all probe specifically associated with its target sequence, notably in the presence of an agent able to denature the probe-target duplex, and at a temperature higher than the melting temperature of the probe under consideration, notably at a temperature of approximately 100°C.

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15. A method according to claim 14, further characterized in that the denaturing agent is formamide.

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16. A method according to any one of the preceding ~~claims~~ ^{Claim 1}, further characterized in that said extracted probes are concentrated, notably using

the Speed-Vac®, prior to the measurement of the amount thereof or of their respective amounts.

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17. A method according to any one of the preceding claims, further
5 characterized in that said detection and amount measurement of extracted probes is performed by detection and amount measurement of a label associated or incorporated into each of the contacted probes, such as a radioactive, chemiluminescent or fluorescent label, notably such as fluorescein.

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18. A method according to any one of the preceding claims, further characterized in that said sample is taken from fluids such as natural water, industrial water, industrial effluent, municipal wastewater, industrial sludge, thermal mud, food liquid or gel, fermentation media, air, gas, aerosol, or is a
15 sample taken from a building ventilation duct, air conditioning duct, sample of food solid, sample of soil, sample from medical apparatus, or is a human or animal sample, such as blood, urine, vaginal or intestinal flora.

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19. A method according to any one of the preceding claims, further
20 characterized in that it is used in combination with a process for triggering an alarm in connection with quality, safety and/or sanitary monitoring of the product from which said sample has been obtained.

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20. A method according to any one of the preceding claims, further
25 characterized in that it is used in *in vitro* diagnosis of an infectious disease.

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21. A method according to any one of the preceding claims, further characterized in that it is used in the automatic or feedback control of a microbiological process such as methane fermentation of liquid manure,

treatment of organic effluents, sewage treatment process such as treatment by activated sludge.

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22. A method according to any of the preceding claims, further characterized in that it is used in the automatic or feedback control of a process relating to the removal or prevention of the development of microorganisms.

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Claim!
23. A method according to any of the preceding claims, characterized in that it is applied in the detection of foam formation during the implementation of activated sludge processes and/or for the feedback control of a method relating to the removal or prevention of the said foams.

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